ATTACHMENT 2

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Effects of atorvastatin and vitamin E on lipoproteins and oxidative stress in dialysis patients: a randomised-controlled trial

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Abstract. Diepeveen SHA, Verhoeven GWHE, van der Palen J, Dikkeschei LD, van Tits LJ, Kolsters G, Offerman JJG, Bilo HJG, Stalenhoef AFH (Isala Clinics, location Weezenlanden, Zwolle; Medical Spectrum Twente, Enschede; and Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands). Effects of atorvastatin and vitamin E on lipoproteins and oxidative stress in dialysis patients: a randomised-controlled trial. *J Intern Med* 2005; 257: 438–445.

Objectives. The objective of this study was to examine the effects of treatment with atorvastatin, α-tocopherol and the combination of both, on lipoproteins and oxidative stress in dialysis patients.

Design and setting. This double-blind randomised placebo-controlled trial was performed at the dialysis department of a non-university hospital.

Subjects, intervention and measurements. A total of 44 clinically stable, non-diabetic patients on dialysis therapy (23 on haemo- and 21 on peritoneal-dialysis) without manifest cardiovascular disease were included in this study. They were randomised for treatment during a period of 12 weeks with 40 mg atorvastatin + placebo α-tocopherol (group 1) once daily, 800 IU α-tocopherol + placebo atorvastatin once daily (group 2), 40 mg atorvastatin + 800 IU α-tocopherol once daily (group 3), or placebo atorvastatin + placebo α-tocopherol once daily (group 4). Assessment of lipid profile and oxidative stress was performed at the start of the study and after 12 weeks of treatment.

Results. Treatment with atorvastatin reduced total cholesterol, triglycerides (TG), low-density lipoprotein (LDL) cholesterol, apolipoprotein B (apoB) and levels of oxidised LDL (oxLDL) with 30–43%. It had no influence on LDL oxidisability. Additional supplementation with α-tocopherol had no effect on lipid profile and oxLDL levels but decreased *in vitro* LDL oxidisability. No side-effects were observed.

Conclusions. Treatment with atorvastatin is effective in lowering plasma total cholesterol, TG, LDL, apoB and oxLDL in a population of stable dialysis patients and might therefore be an effective tool in improving the poor cardiovascular outcome in these patients. Supplementation of α-tocopherol to atorvastatin had beneficial effects on *in vitro* LDL oxidisability and might therefore be of additional value. Further research on the clinical effects of treatment with atorvastatin in combination with α-tocopherol is necessary.

Keywords: dialysis, lipoproteins, oxidative stress, randomised-controlled trial, statin, vitamin E.

Introduction

Cardiovascular disease (CVD) is the most important cause of morbidity and mortality in patients with end-stage renal disease (ESRD) treated with maintenance dialysis. Patients with ESRD have a highly atherogenic lipid profile and increased levels of oxidised LDL (oxLDL)

which are associated with atherosclerosis and CVD [1, 2].

Treatment of dyslipidaemia in non-renal patients with statins has been shown to be effective in improving the lipid profile, reducing cardiovascular events and improving survival [3]. In dialysis patients, treatment with statins also appeared to be save and effective in improving lipid abnormalities [4]. Furthermore, statins were shown to have beneficial effects on the oxidisability of LDL and circulating oxLDL levels [5]. An observational study in a cohort of dialysis patients showed that the use of statins was associated with reduced mortality [6]. However, data on clinical end-points from prospective, randomised-controlled trials of statins in patients with ESRD are lacking.

As ESRD and dialysis therapy are associated with enhanced oxidative stress and this is thought to play an important role in atherogenesis, patients with ESRD and on dialysis therapy might benefit from antioxidant therapy. Supplementation with vitamin E in populations with and without renal disease indeed has beneficial effects on oxidative stress and (surrogate) markers of atherosclerotic disease but the results on clinical end-points in general have been disappointing. A recent meta-analysis on this subject concluded that there is no indication for routine use of antioxidants in order to improve cardiovascular outcome [7]. However, most studies are performed in non-renal populations and the only prospective study on secondary prevention with antioxidants in dialysis patients, reported a reduction in composite cardiovascular end-points [8]. In order to try to understand the effects of intervention with statins and vitamin E, we performed a prospective, double-blind, randomised placebo-controlled trial of treatment with atorvastatin and α-tocopherol in dialysis patients and studied the effects on lipoproteins and oxidative stress.

Materials and methods

Study population and medication

We studied 44 clinically stable non-diabetic patients on dialysis therapy [23 on haemodialysis (HD) and 21 on peritoneal dialysis (PD)] without manifest CVD. None of the patients used lipid-lowering drugs. All patients were treated in the dialysis unit of the Isala Clinics, location Weezenlanden.

Patients were randomised for treatment with once daily 40 mg atorvastatin + placebo α-tocopherol (group 1), once daily 800 IU α-tocopherol + placebo atorvastatin (group 2), once daily 40 mg atorvastatin + once daily 800 IU α-tocopherol (group 3), or once daily placebo atorvastatin + placebo α-tocopherol (group 4).

All patients gave their informed consent. The hospital's Medical Ethical Committee approved of the study.

Parke-Davis/Pfizer® (Capelle aan den IJssel, the Netherlands) supplied the atorvastatin and placebo tablets. The hospital's pharmaceutical department manufactured capsules with α-tocopherol and placebo.

Laboratory measurements

Blood sample collection. Blood samples were taken before the start of the study and after 12 weeks of treatment. Patients were instructed to take a light, low fat containing meal, consisting of tea and a jelly sandwich, on the day of the blood sample collection. In the HD-patients, blood samples were taken just before a dialysis session. Venous blood was collected in ethylenediaminetetraacetic acid (EDTA) Vacutainer® tubes (Becton Dickinson, Heidelberg, Germany). The blood samples were immediately placed on ice and plasma was obtained by centrifugation (1500 *g*) at 4 °C within 30 min. Plasma, supplemented with saccharose (0.6%, w/v) as a cryoprotectant, was frozen on liquid nitrogen and stored at −80 °C until analysis.

Lipid profile, creatinin, blood urea nitrogen, haemoglobin and apolipoprotein B. Plasma total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), creatinin and blood urea nitrogen (BUN), were measured on a Hitachi 917 analyser, using Roche reagents (Roche, Mannheim, Germany). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula. Haemoglobin (Hb) was measured on a Sysmex XE-2100 (Toa Medical Instruments, Kobe, Japan).

Apolipoprotein B (apoB) was analysed on the Hitachi 912 chemistry analyzer using Roche reagents (Roche, Almere, the Netherlands).

LDL-oxidisability. After thawing of the plasma sample, LDL was isolated by density-gradient

ultracentrifugation (40 000 rpm in a SW 40 rotor for 18 h at 4 °C; Beckman, Palo Alto, CA, USA). The protein content of the LDL fraction was measured and LDL oxidation assays were performed as described by Kleinveld *et al.* [9]. Briefly, the oxidation of LDL (60 μg apolipoprotein mL^{-1}) was initiated by the addition of $CuSO_4$ (final concentration of 18 μmol L^{-1}) at 37 °C. The kinetics of the oxidation of LDL was determined by monitoring the change of the 234 nm diene absorption in a water-thermostated UV spectrophotometer (Lambda 12, Perkin-Elmer, GmBH, Germany), equipped with an 8-position automatic row-sampler changer. Each LDL preparation was oxidised twice, in two separate oxidation runs on the same day. Every oxidation run was controlled by analysing one reference LDL, freshly prepared from pooled plasma stored with 6 g L^{-1} saccharose at −80 °C.

Oxidised LDL. Plasma oxLDL was measured using the Mercodia oxLDL kit (Mercodia©, Uppsala, Sweden). This immunoassay is based on the direct sandwich technique and uses two monoclonal antibodies that are directed against separate antigenic determinants on the oxidised apoB molecule. The intra- and inter-assay coefficient of variation amount to 6 and 7% respectively.

Vitamin E. Concentrations of vitamin E in serum and LDL were analysed by reversed phase high-performance liquid chromatography.

Statistical analysis

Statistical analysis was performed using SPSS 11.0 for Windows. Values are expressed as mean ± SD.

The Wilcoxon signed rank test was used to compare differences in continuous variables within groups after intervention. One-way ANOVA with Bonferroni correction was performed to compare baseline differences between groups. *P*-values ≤0.05 were considered statistically significant.

Results

Population

Demographic characteristics of the study population are presented in Table 1. There were no significant differences between the four groups, although groups 1 and 4 have been subjected longer to dialysis therapy than groups 2 and 3. Patients experienced no side-effects of the medication during the study period.

Separate analysis of HD- and PD-groups showed comparable results and therefore the results of the interventions are described together.

Lipid profile

Baseline values of total cholesterol, TG, HDL cholesterol, LDL cholesterol and apoB did not differ between the groups (Table 2a–d).

Treatment with atorvastatin (group 1) reduced total cholesterol, TG, LDL cholesterol and apoB with 30–43%. These values were not influenced by additional supplementation with α-tocopherol (group 3). Supplementation with α-tocopherol alone had no effect on the lipid profile (group 2).

Vitamin E and oxidative stress

Baseline values of lag-time, oxLDL, serum vitamin E and vitamin E per mg LDL protein, did not differ between the groups (Table 2a–d). Whilst serum vitamin E and vitamin E per mg LDL protein were comparable with values of normal subjects (26.3 ± 3.6 mg L^{-1} plasma and 14.1 ± 1.2 mg g^{-1} LDL protein, respectively) [10], lag-time of dialysis patients surprisingly was longer than in normal subjects (70.4 ± 11.1 min) [11]. OxLDL was slightly lower in dialysis patients than in normal subjects (median 59 U L^{-1}, range: 29–117, data supplied by Mercodia), which may be due to the relatively low LDL levels. Normal values for oxLDL/apoB ratio are not known.

Before intervention, a positive correlation was found between oxLDL and vitamin E in serum (correlation coefficient: 0.542, $P < 0.01$) and between oxLDL and vitamin E in LDL (correlation coefficient: 0.345, $P < 0.01$) in the overall group. A negative correlation between lag-time and vitamin E in LDL (correlation coefficient: −0.511, $P < 0.01$) was found in this group of patients.

After treatment with α-tocopherol (groups 2 and 3) the concentrations of vitamin E in plasma and in LDL were increased. The degree of increase did not differ between both groups.

In addition to its effects on lipid profile, atorvastatin (group 1) reduced the plasma level of oxLDL

Table 1 Baseline demographic and treatment characteristics of the dialysis patients by groups

Randomisation*	Group 1 (n = 13)	Group 2 (n = 10)	Group 3 (n = 11)	Group 4 (n = 10)
Male : female	9 : 4	8 : 2	5 : 6	8 : 2
Age (years)	46 ± 15	47 ± 16	51 ± 20	51 ± 18
Duration of dialysis (months)	118 ± 107	55 ± 31	56 ± 30	118 ± 79
Systolic blood pressure (mmHg)	142 ± 30	153 ± 26	147 ± 21	156 ± 33
Diastolic blood pressure (mmHg)	87 ± 16	93 ± 18	89 ± 13	91 ± 15
Body mass index (kg m^{-2})	24.6 ± 4.6	23.5 ± 2.8	24.5 ± 3.1	26.0 ± 5.4
Hb (mmol l.$^{-1}$)	7.0 ± 0.8	7.2 ± 0.6	7.4 ± 1.2	7.1 ± 1.0
Creatinin (μmol L^{-1})	1077 ± 350	916 ± 187	953 ± 261	1071 ± 183
Blood urea nitrogen (mmol L^{-1})	23.4 ± 5.5	27.4 ± 4.5	24.9 ± 5.3	26.0 ± 3.8
Antihypertensive drugs ≥1	3 (23%)	4 (40%)	3 (27%)	7 (70%)
Phosphate-binding drugs ≥1	13 (100%)	10 (100%)	>11 (100%)	10 (100%)
Dialysis treatment				
Weekly HD duration (h)	11.8 ± 1.1	12.5 ± 1.6	10.5 ± 1.0	12.0 ± 2.4
Daily PD treatment (L)	10.5 ± 2.6	8.1 ± 2.7	9.2 ± 1.8	10.6 ± 2.5

Values are expressed as mean ± SD.
No differences were found between the four groups regarding the described characteristics.
*Randomisation: group 1, atorvastatin + placebo; group 2, α-tocopherol + placebo; group 3, atorvastatin + α-tocopherol; group 4, placebo + placebo.
Hb, haemoglobin.

and the concentration of vitamin E, but had no effect on vitamin E in LDL, the LDL oxidisability and the ratio oxLDL/apoB. Additional suppletion of α-tocopherol did not influence the effect of atorvastatin on oxLDL but led to a reduction of LDL oxidisability (group 3). Furthermore, a small increase in oxLDL/apoB ratio was found. Treatment with α-tocopherol alone (group 2) also prolonged the lag-time by 13% (P = 0.051). The concentration of oxLDL in plasma was increased after supplementation with α-tocopherol alone but the oxLDL/apoB ratio did not change (group 2).

Discussion

In the present study, we investigated the effects of treatment with atorvastatin in combination with the antioxidant α-tocopherol on biochemical variables of lipid profile and oxidative stress, in order to gain insight in mechanisms underlying the beneficial effects of these treatments in earlier studies.

We observed marked decreases in the concentrations of total cholesterol, TG, LDL-C, apoB and circulating oxLDL particles and a minimal decrease in serum vitamin E after treatment with atorvastatin (40 mg daily) in stable dialysis patients. Intervention with α-tocopherol (800 IU daily) raised serum vitamin E and vitamin E in LDL, prolonged the lag-time of LDL oxidation but also was associated with increased levels of oxLDL. Treatment with α-tocopherol in combination with atorvastatin showed a decrease of concentration of lipids and oxLDL and also a prolonged lag-time of LDL oxidation.

Epidemiology and risk factors of cardiovascular disease in renal patients

Enhanced atherosclerosis and consequent increased cardiovascular morbidity and mortality are important clinical problems in patients with renal disease on dialysis. Mortality from CVD in dialysis patients is about 9% per year. This is about 30 times higher than in the general population [12].

The Framingham Heart Study established the traditional risk factors for developing CVD. Most of these traditional risk factors (age, diabetes mellitus, hypertension, left ventricular hypertrophy, low concentrations of HDL-C) are also present in patients on dialysis treatment and contribute to

Table 2 Results of treatment with (a) atorvastatin + placebo α-tocopherol (group 1, $n = 13$). (b) α-tocopherol + placebo atorvastatin (group 2, $n = 10$). (c) atorvastatin + α-tocopherol (group 3, $n = 11$) and (d) placebo atorvastatin + placebo α-tocopherol (group 4, $n = 10$) in dialysis patients

	Baseline	12 weeks	Mean change (%)	*P*-value
(a) Atorvastatin + placebo α-tocopherol (group 1, $n = 13$)				
Cholesterol (mmol L^{-1})	5.1 ± 1.1	3.3 ± 0.9	−34	0.003
TC (mmol L^{-1})	3.8 ± 3.3	2.4 ± 1.9	−34	0.003
HDL-C (mmol L^{-1})	0.97 ± 0.3	0.94 ± 0.3	−1.9	0.834
LDL-C (mmol L^{-1})	2.6 ± 0.9	1.3 ± 0.8	−45	0.016
ApoB (g L^{-1})	1.0 ± 0.2	0.7 ± 0.2	−30	0.003
Lag-time (min)	93 ± 21	94 ± 20	1.9	0.388
OxLDL (U L^{-1})	46 ± 13	33 ± 11	−29	0.001
Ratio oxLDL/apoB (U g^{-1})	47 ± 12	48 ± 9.6	5.6	0.507
Serum vitamin E (μmol L^{-1})	36 ± 15	30 ± 13	−4.8	0.023
Vitamin E in LDL (μmol g^{-1} LDL protein)	16 ± 4.2	15 ± 3.9	−5.5	0.117
(b) α-Tocopherol + placebo atorvastatin (group 2, $n = 10$)				
Cholesterol (mmol L^{-1})	4.8 ± 1.1	4.8 ± 1.0	1.9	0.798
TG (mmol L^{-1})	2.5 ± 1.5	2.3 ± 1.4	−4.2	0.477
HDL-C (mmol L^{-1})	0.95 ± 0.3	0.98 ± 0.3	4.8	0.635
LDL-C (mmol L^{-1})	2.7 ± 0.9	2.8 ± 1.0	4.4	0.445
ApoB (g L^{-1})	1.0 ± 0.3	1.1 ± 0.3	5.8	0.114
Lag-time (min)	86 ± 17	98 ± 17	13	0.051
OxLDL (U L^{-1})	41 ± 9.3	49 ± 14	17	0.015
Ratio oxLDL/apoB (U g^{-1})	42 ± 8.2	44 ± 7.5	9.8	0.110
Serum vitamin E (μmol L^{-1})	27 ± 4.6	51 ± 18	88	0.008
Vitamin E in LDL (μmol g^{-1} LDL protein)	15 ± 2.7	21 ± 6.2	42	0.012
(c) Atorvastatin + α-tocopherol (group 3, $n = 11$)				
Cholesterol (mmol L^{-1})	4.9 ± 1.2	3.1 ± 0.8	−37	0.003
TG (mmol L^{-1})	1.7 ± 1.0	1.4 ± 0.7	−9.9	0.047
HDL-C (mmol L^{-1})	1.46 ± 0.2	1.10 ± 0.2	−3.7	0.169
LDL-C (mmol L^{-1})	3.0 ± 0.9	1.4 ± 0.6	−55	0.003
ApoB (g L^{-1})	1.1 ± 0.2	0.7 ± 0.2	−35	0.004
Lag-time (min)	88 ± 9.1	104 ± 12	19	0.003
OxLDL (U L^{-1})	44.8 ± 8.4	32.8 ± 9.8	−28	0.004
Ratio oxLDL/apoB (U g^{-1})	42.6 ± 4.6	47.7 ± 4.2	13	0.021
Serum vitamin E (μmol L^{-1})	28.1 ± 9.3	40.1 ± 11	46	0.004
Vitamin E in LDL (μmol g^{-1} LDL protein)	14.3 ± 2.0	23.3 ± 5.3	64	0.01
(d) Placebo atorvastatin + placebo α-tocopherol (group 4, $n = 10$)				
Cholesterol (mmol L^{-1})	4.8 ± 1.1	4.9 ± 0.8	4.2	0.609
TG (mmol L^{-1})	2.6 ± 1.7	2.3 ± 1.2	−1.3	0.445
HDL-C (mmol L^{-1})	0.92 ± 0.3	0.92 ± 0.3	−0.8	0.878
LDL-C (mmol L^{-1})	2.7 ± 0.8	3.0 ± 0.8	12	0.028
ApoB (g L^{-1})	1.0 ± 0.3	1.1 ± 0.3	8.9	0.126
Lag-time (min)	90 ± 13	89 ± 11	−0.7	0.683
OxLDL (U L^{-1})	42 ± 12	43 ± 10	3.7	0.646
Ratio oxLDL/apoB (U g^{-1})	41 ± 7.8	39 ± 7.3	−4.4	0.241
Serum vitamin E (μmol L^{-1})	29 ± 11	31 ± 11	5.7	0.333
Vitamin E in LDL (μmol g^{-1} LDL protein)	14 ± 2.7	15 ± 3.2	9.8	0.326

Values are expressed as mean ± SD.
apoB, apolipoprotein B; oxLDL, oxidised low-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride.

the high incidence of CVD in this population. Because of the large difference in cardiovascular mortality between the general and the dialysis population, it is suggested that in renal failure aggravation of traditional cardiovascular risk factors and/or additional cardiovascular risk factors are present. The cardiovascular risk factors recognised in dialysis patients are, amongst others: anaemia, chronic inflammation, malnutrition, hyperhomocysteinaemia, hyperparathyroidism, hyperphosphataemia, dyslipidaemia and enhanced oxidative stress [13]. Two risk factors for CVD, i.e. dyslipidaemia and enhanced oxidative stress, will be discussed further.

Dyslipidaemia in renal disease

Dialysis patients suffer from a disturbed lipid metabolism and this contributes to the enhanced atherosclerosis and the increased cardiovascular mortality in this population [2]. Dyslipidaemia in this population is characterised by abnormal composition of the lipoproteins and the apolipoprotein profile is disturbed: the concentration of HDL-C is mostly low whereas concentrations of TG and/or very low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL) are increased [2, 14]. Hypertriglyceridaemia is associated with the presence of small dense LDL. This LDL subfraction is believed to be highly atherogenic. The precise mechanism of this metabolic disturbance is not fully understood, but amongst others, a defective catabolism of TG-rich lipoproteins by lipoprotein lipase and hepatic lipase are believed to play a role in this process [15]. Several authors describe the clinical relevance of dyslipidaemia in this population. Hocher *et al.* demonstrated in a HD population that low concentrations of HDL-C were associated with all-cause mortality [16]. Shoji *et al.* identified IDL-C, included as part of the LDL-C, as independent risk factor for aortic atherosclerosis [17].

Effects of treatment with statins

The treatment of hypercholesterolaemia with statins has been proven to be effective and safe and associated with beneficial effects on mortality in large interventional trials [3]. As in these trials patients with severe renal impairment often were excluded, data on effects of statins on clinical end-points in these patients are scarce. In an observational study, Seliger *et al.* found that in a cohort of HD patients the use of a statin was associated with a 32% lower mortality risk [6]. Several small, randomised-controlled trials with statins on biochemical end-points have been performed in dialysis patients [4, 5]. The results of these studies are promising: the treatment of dialysis patients with statins is safe and effectively improves lipid profile. Despite the fact that the baseline values of total cholesterol and lipoproteins were different, the proportional degree of lipid reduction was similar in all studies and this also agrees with our present findings. In our study, baseline values of total cholesterol and LDL-C were in the normal range, but treatment with atorvastatin still effectively lowered the concentrations of total cholesterol, TG, LDL-C and improved the concentration of apoB. However, the beneficial effects on CVD by treatment with statins may not only be determined by the absolute reduction of total cholesterol, but also by the reduction of the highly atherogenic lipoproteins, including oxLDL particles. OxLDL is more atherogenic than native LDL: it is immunogenic, has proinflammatory effects and is excessively taken up by macrophages, leading to foam cell formation. We found that the levels of oxLDL were decreased by treatment with atorvastatin and suggest that this reduction possibly can contribute to a lesser degree of atherosclerosis. In agreement with this, treatment with statins was found to be associated with lower levels of C-reactive protein, reflecting a lower state of chronic inflammation [18].

Based on the results of these studies, treatment of renal patients with statins might be a promising tool to improve the cardiovascular outcome in these patients. Still convincing evidence is lacking for application of these drugs on large-scale in this population. Large randomised-controlled trials with statins on cardiovascular morbidity and mortality in dialysis patients are underway and these studies will hopefully tell us unequivocally whether and when to treat dialysis patients with statins or not.

Oxidative stress in dialysis patients

In the pathogenesis of atherosclerosis, oxidative changes in lipoproteins are supposed to play a pivotal role. Enhanced oxidative stress is therefore considered as one of the so-called novel risk factors of CVD. As dialysis is associated with oxidative stress, this therapy may seriously enhance atherogenesis in this population. It might therefore be worthwhile to search for interventional strategies to reduce the amount of oxidative stress.

Treatment with antioxidants

Large interventional studies in different populations (both primary and secondary prevention) with antioxidant therapies have been performed trying to improve outcome on cardiovascular morbidity and mortality. However, the results of these studies are conflicting. The Cambridge Heart Antioxidant Study (CHAOS) studied the effects of

treatment with vitamin E in patients without renal disease [19]. A decreased rate of myocardial infarction was observed after treatment with vitamin E, although there was no effect on mortality. In the Antioxidant Supplementation in Atherosclerosis Prevention Study (ASAP) it was demonstrated that supplementation with a combination of vitamins E and C delayed the progression of atherosclerosis, as determined by ultrasonic measurement of the intima media thickness of the common carotid artery in hypercholesterolaemic patients [20]. No effects of both vitamins were observed with respect to F2-isoprostanes, a parameter of oxidative stress. In contrast to these 'positive' studies, the Heart Protection Study [21] and Heart Outcomes Prevention Evaluation Study [22] did not demonstrate any beneficial influence of intervention with respectively multivitamin antioxidant supplementation (vitamin E, vitamin C and β-carotene) and vitamin E on clinical end-points. In the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI)-Prevenzione trial, in which patients with a recent myocardial infarction were included, treatment with vitamin E also had no beneficial effects on cardiovascular end-points [23].

The only prospective study with clinical end-points on vitamin E in a dialysis population so far was performed in HD patients with manifest CVD (SPACE) [8]. It showed that supplementation with vitamin E had beneficial effects on composite CVD end-points and myocardial infarction. More interventional studies have been carried out with supplementation of vitamin E on biochemical end-points. The results of these studies are not unequivocal: some show beneficial effects on parameters of oxidative stress, whereas others do not. The different studies however, are hard to compare because of the use of different markers of oxidative stress. Beside studies with vitamin E supplementation, studies with vitamin E-coated membranes have been performed in HD patients. The results of these studies point to a reduction in oxidative stress and possibly an improvement of atherosclerosis. Prospective studies using these vitamin E-coated membranes on clinical end-points are lacking.

In the present study, we demonstrate that supplementation with 800 IU α-tocopherol daily resulted in a nearly significant increase of resistance of LDL-C to oxidation *in vitro* as reflected by increased lag-time. On the contrary, treatment with α-tocopherol slightly increased the concentration of oxLDL. This increase in oxLDL might be related to a small, insignificant increase in LDL-C and/or to a pro-oxidative effect of α-tocopherol that can be mediated by deficiency of co-antioxidants [24]. Because the oxLDL/apoB ratio remained unchanged, negative effects of α-tocopherol on atherogenesis are unlikely. Combination therapy of atorvastatin and α-tocopherol led to an increase in LDL lag-time and a significant reduction in circulating oxLDL. As the latter effect is also seen in patients treated with atorvastatin only, this results most likely from the reduction of plasma LDL. Relative changes in oxLDL in atorvastatin-treated patients were independent of co-addition of α-tocopherol, suggesting that a pro-oxidative effect of α-tocopherol does not occur at low concentrations of LDL-C. The slight increase in the oxLDL/apoB ratio that we found in patients treated with atorvastatin and α-tocopherol is most likely the result of a larger decrease of apoB compared with the decrease of oxLDL.

From our study we conclude that treatment with 40 mg atorvastatin once daily is effective in lowering plasma total cholesterol, TG, LDL, apoB and oxLDL in a population of stable dialysis patients. No side-effects were observed at this rather high dose in this population. These changes in the lipid profile can possibly contribute to improvement of the poor cardiovascular outcome of these patients. Ongoing large clinical trials will have to provide the conclusive evidence for this. Supplementation of α-tocopherol alone did enhance resistance to *in vitro* oxidation of LDL-C, but on the other hand, was associated with a slight elevation in plasma oxLDL levels. Although a causal relation between the elevation of circulating oxLDL and the use of vitamin E is unlikely, the clinical benefit of α-tocopherol supplementation alone is questionable. However, in combination with a statin, no increase in plasma oxLDL levels occurred and therefore additional α-tocopherol supplementation in combination with a statin may be beneficial because of the decrease of LDL oxidisability.

Conflict of interest statement

No conflict of interest was declared.

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